[3] H. Shimizu et al., Cell 132:67-78 (2008)
[4] T. Kanzaki et al., J. Biol. Chem. 283:34773-34784

**973-Pos**

**Optimal Estimation of the Diffusion Coefficient from Noisy Time-Series Measurements**

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Single-molecule time-lapse measurements of diffusing proteins often contain considerable localization error. The standard method for estimating the diffusion coefficient is based on the mean square displacements. This method is highly inefficient, since it ignores the high correlations inherent to these. A Generalized least squares method, which takes into account these correlations, is presented and it is shown that it attains the maximum precision possible according to information theory. The method is demonstrated on data from high-speed time-lapse photography of the hOgg1 repair protein diffusing on DNA.

**974-Pos**

**Adaptive Platform for Highly Parallel Low-Noise Recordings of Single Membrane Proteins**

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Highly parallel, low noise electrophysiological recordings of single ion channels are of interest both for basic research and drug development. Here, a microsystems approach is presented (see Fig.1A) which greatly simplifies the recording configuration and optimizes the electrical parameters governing noise. The lipid bilayer is formed on a picoliter cavity generated within a photochemical resist acting as a dielectric (see Fig. 1B). On the bottom of each cavity a microelectrode is placed.

Using standard photolithographical techniques this design allows for many such setups on one chip, and is therefore in principle well suited for highly parallel single channel recordings. Parallel recordings (16-electrode multiarray) of currents mediated by alamethicin are shown (see Fig.1C), illustrating the potential of this novel approach towards high-throughput measurements of single membrane proteins. Furthermore, the flexibility of this approach is outlined by the reconstitution of α-hemolysin in the lipid bilayer. The capability for molecular analysis is demonstrated by the detection of oligomers diffusing through the bacterial pores.

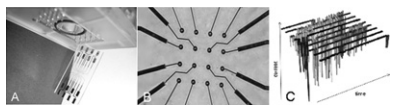


**Fig. 1.** *Patch16Chip* ***(B)*** *Connecting lines and cavities over the circular shaped electrodes.* ***(C)*** *Current traces of single alamethicin-channels, detected in parallel at 9 different cavities.*

**975-Pos**

**A New Closed Cell, Horizontal Magnetic Tweezer**

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We report on the development of a magnetic force transducer or tweezer that can apply piconewton forces on single DNA molecules in the focus or horizontal plane. Since changes in the DNA's end-to-end extension are coplanar with the pulling force there is no requirement to continually refocus the tethered beads thus considerably simplifying single molecule micromanipulation experiments in our setup. The DNA constructs (γ-DNA end-labeled with a 3μm polystyrene bead and a 2.8μm magnetic sphere) and buffer are introduced into a 200μL to 500 μL closed cell created by using two glass slides separated by 1mm spacers and a thin viscoelastic perimeter wall. This closed cell configuration isolates our sample and produces low-noise force and extension measurements. Breaching the viscoelastic barrier are five pipettes: a 1-2μm inner diameter suction pipette used for capturing the polystyrene bead, a magnet-tipped pipette used to pull the magnetic sphere, a 0.5mm-inner-diameter injection pipette used to introduce proteins of interest, and two 0.5mm-inner-diameter pipettes used to maintain flow. The suction micropipette and the injection pipette are rigidly coupled and positioned by a manual three axis manipulator that can produce continuous displacements of 15mm, 25mm and 25mm in the x-, y-, and z-axis respectively. The magnet-tipped pipette is controlled by a motorized two axis micromanipulator capable of continuously spanning the full width of the cell. A motorized micromanipulator with a defined 157nm step size maneuvers the cell over a 32X/0.40NA objective and between the two mechanical manipulators. Initial tests show the capability of the device to easily and repeatedly find, capture, and manipulate end-labeled DNA constructs.

**976-Pos**

**Optical Trapping and Two-Photon Exitations of Quantum Dots**

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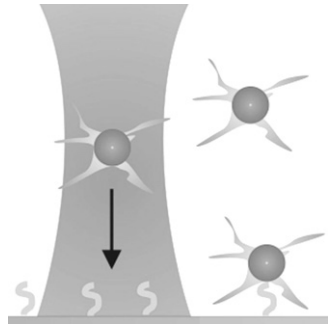
Individual colloidal quantum dots can be optically trapped and manipulated by a single infrared laserbeam operated a low laser powers [Jauffred et al. Nano letters 2008 (**10**)] and the spring constant of the force, exerted by the harmonic optical trap on a quantum dot, have been found to be of the order of $10^{-4}$ pN/nm. We measured the optical trapping strength of individual colloidal quantum dots with different emission wavelenghts (from 525 nm to 800 nm) and different physical sizes, with the result that these diverse quantum dots have identical trapping capabilities.



Furthermore, we show that the trapping laserlight can also act as a source for two-photon excitation of the trapped quantum dots, thus eliminating the demand for an excitation light source in addition to the trapping laser beam.

**977-Pos**

**Optical Torque Wrench for Single Molecule Studies**

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At the molecular level, the torque applied to biopolymers plays a central role in many processes involving their conformational changes and interactions with proteins. We will study the torque-sensitivity of individual nucleic acid molecules and their interactions with proteins using a novel optical tweezers configuration termed the optical torque wrench [1].

In standard single-molecule techniques, torque cannot be simultaneously controlled and detected, in contrast with the case of the applied force. With this new technique we will be able to control both parameters in real-time in single molecules of DNA or RNA, with high temporal (250 kHz) and spatial (nm) resolution typical of optical tweezers. Here we present a first characterization of our instrument.

This will allow us to acquire fundamental insight into the torque-sensitivity and dynamics of nucleic acids, DNA packaging, polymerase activity in DNA replication or transcription, and related biological processes.

[1] A. La Porta and M.D.Wang, Phys. Rev. Lett. 92, 190801, 2004.

## Atomic Force Spectroscopy

**978-Pos**

**Fishing on Living Cells with AFM: Novel Method to Study Topology and Dynamics of Cotransporter SGLT1 Protein**

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In the apical membrane of epithelial cells from the small intestine and the kidney, the high-affinity $Na^+$/D-glucose cotransporter SGLT1 plays a crucial role in intestinal glucose absorption and in renal glucose reabsorption. Here the over-expression of rabbit SGLT1 in rbSGLT1-transfected Chinese hamster ovary (CHO) cells was first characterized using the immuno-staining method on non-permeabilized cells. The functionality of the SGLT1 was verified by biochemical approaches. Atomic force microscopy (AFM) was employed to probe initial substrate-carrier interactions, topology, and function of SGLT1 in living cells on the single-molecule level. Specific recognition events in force distance cycles were detected using epitope specific antibodies or thio-glucoside bound to the AFM tip. Upon addition of D-glucose or the specific inhibitor phlorizin, binding of antibody primed AFM tips drastically decreased suggesting recognition sites for D-glucose in the extracellular loop 8-9 and for phlorizin in the extracellular loop 13-14. The binding probability of the thio-D-Glucose tip was reduced by various sugars in a potency sequence that differed markedly from transport studies. We therefore propose that the first of several selectivity filters of SGLT1 is formed by the two extracellular loops 8-9 and

13-14. This region undergoes significant conformational changes during sugar translocation. Moreover, the results from the thio-D-glucose coupled via a long heterobifunctional crosslinker with a small end group showed the possibility to observe the D-glucose transport pathway of the SGLT1. These studies demonstrate that AFM is a powerful method to explore the structural and functional dynamics of plasma membrane transport proteins in live cells on a single molecule level.

**979-Pos**

**Simultaneous Topography and Recognition (TREC) of Proteins in the Pathological Deposits in Pseudoexfoliation Syndrome using AFM**

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Protein aggregation is of significant interest to various disciplines; it can be the cause of debilitating diseases, or the foundation of advanced nanomaterials. One ocular disease hallmarked by protein aggregation is known as Pseudoexfoliation Syndrome (PEX). This condition is caused by the formation of insoluble aggregates, and is characterised by deposition of fibrillar proteinaceous material on the anterior lens capsule. PEX deposits in the eye block the aqueous outflow mechanisms, which can lead to an elevation in intraocular pressure and subsequent glaucoma. Glaucoma is the second leading cause of irreversible blindness worldwide, and PEX is the most common known risk factor for glaucoma.

Proteomic analyses have revealed an association of various genetic markers and protein expression with PEX; however a complete explanation for disease susceptibility is not known. As the aggregates are a complex arrangement of proteins, the ultrastructure is poorly characterised and many protein constituents of the aggregates remain unknown. This study addresses the critical issue of determining the molecular nature of PEX on lens capsules in their native state by atomic force microscopy (AFM) based antibody recognition imaging. This AFM methodology is referred to as Topography and RECognition imaging (TREC). Proteins identified as being implicated in the PEX pathophysiology are detected by an AFM probe modified with the appropriate antibody. Topographical AFM images and antibody recognition images are obtained simultaneously to determine the specific location of proteins in and around PEX aggregates. This data, combined with data from proteomic and genetic analyses, is leading to an improved understanding of the pathophysiological basis of PEX. A more complete understanding of the pathophysiological basis for the disease will lead to earlier detection methods and treatments that target the disease instead of the subsequent glaucoma.

**980-Pos**

**Deciphering Podosome Physical Properties in Human Macrophage by Atomic Force Microscopy**

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Macrophages belong to phagocytes which constitute the first line of host defense. Directed migration and adhesion of these cells through anatomic boundaries is crucial for their functions. Moreover, tissue infiltration of macrophages has been shown to worsen many pathologies such as atherosclerosis, chronic inflammation and cancer. Our research focuses on understanding the adhesive and motile behaviors of macrophages with particular emphasis on podosomes, dynamic actin-rich structures only found in macrophage and macrophage-derived cells required for normal adhesion and migration. Although the minimal structural feature of podosome can be defined as an F-Actin-rich core surrounded by a ring containing proteins such as Vinculin and Integrins, the mechanisms involved in podosome biogenesis and architecture are poorly understood. Thanks to the atomic force microscope operated in liquid, it is now possible to explore cells at the nanoscale in terms of topographic and force measurement mode. Here we choose to use this powerful technique to explore the mechanisms involved in biogenesis, bio-physical properties and architecture of podosome in human monocyte-derived macrophage. Micro-contact printing technique was used to generate patterns of different physiological extra-cellular matrix (ECM) proteins in order to delineate podosome formation in vitro and investigate the influence of the nature of the substrate on their bio-physical properties knowing that the molecular recognition of ECM protein inducing podosome formation involved an integrin-dependant signaling. Our preliminary experiments using AFM allowed us to measure the height, the dynamic and the Young's modulus of podosomes in different situations. We will present our last results of this ongoing work.

**981-Pos**

**Geometric Influences on Radial Indentation of Microtubules**

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Microtubules assembled in vitro exist in a variety of configurations that vary in number of protofilaments, radius, and skew angle of protofilaments relative to the main microtubule axis. Such variations affect microtubule stability, energetics, and assembly/disassembly dynamics. Further, the most abundant microtubule geometries observed in vitro are influenced by assembly conditions and stabilization methods. We have studied the relationship between microtubule geometry and mechanical properties using finite element modeling (FEM). Specifically, we have examined the effects of protofilament number, microtubule radius, and protofilament skew on the radial stiffness (effective radial spring constant of the microtubule wall) of microtubules as measured in atomic force microscopy (AFM) experiments. Our previous AFM work determined that microtubules assembled in the presence of a slowly-hydrolysable GTP analog, GMPCPP, have enhanced radial stiffness relative to those stabilized with paclitaxel. We surmise that in vitro populations of GMPCPP-microtubules and paclitaxel microtubules contain distinct distributions of microtubule geometries, so we have used FEM to examine the relative effect of microtubule geometry on stiffness values we measure. Our modeling results indicate that the changes in stiffness that we have observed experimentally are not simply a result of changes in protofilament number or orientation but instead are likely due to a relative change in material properties (e.g. effective Young's modulus) of the tubulin polymers.

**982-Pos**

**Organization of RAG1/2 and RSS DNA in the Post-Cleavage Complex**

**Svetlana Kotova**[1], Gabriel J. Grundy[2], Santiago Ramon-Maiques[2], Emilios K. Dimitriadis[1], J. Bernard Heymann[3], Alasdair C. Steven[3], Martin Gellert[2], Wei Yang[2].
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V(D)J recombination is central to establishing a functional adaptive immune system. The large repertoire of immunoglobulins and T-cell receptors is generated by combinatorial rearrangement of an extensive array of variable (V), diversity (D), and joining (J) gene segments that are joined to encode the variable domains of the protein chains. The recombination signal sequences (RSS) that flank these gene segments are recognized, paired in a synaptic complex, and cleaved by collaboration of the lymphoid-specific proteins RAG1 and RAG2. After cleavage, the signal ends remain tightly bound to the RAG proteins in a particularly stable Signal-End Complex (SEC).

To obtain 3D structural information about RAG1/2 bound to RSS DNA, isolated and purified SEC were visualized by AFM. To better define the arrangement of the RAG proteins and RSS DNA in the complex, we used RAG1 and RAG2 fused with maltose binding protein (MBP). A wide variety of complex shapes was recorded, however, it was clear that the two DNA chains predominantly exited the SEC complex from adjacent points. The volume of the protein core was consistent with the expected mass of 500 kDa corresponding to (RAG1)2-(RAG2)2 composition. MBP protrusions could be observed on the protein particles marking the N-termini of RAG1 and RAG2. To make their appearance more noticeable, we used selective antibody labeling. Fab-labeled MBPs were clearly identified peripheral to the recombinase core. When only the RAG2 MBPs were labeled, the two DNAs most often exited together from the SEC on the opposite side to the Fabs. Consistent with this observation, when only the RAG1 MBPs were labeled, they were situated closer to the exiting DNAs.

The parallel arrangement of DNA and protein subunits found by AFM is in an excellent agreement with the 3D model based on EM data.

**983-Pos**

**Nanoscale Tissue Scaffold Investigations to Optimize Central Nervous System Prosthetic**

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The introduction of scaffolding materials with appropriate biochemical cues and physical properties into damaged sites within the central nervous system can encourage endogenous or exogenous cellular re-colonization. The scaffolding material currently under investigation is a synthetic electrospun polyamide